

CLAIMS:

1. A method for characterising a population of polypeptides, which method comprises:
 - (a) contacting a sample comprising polypeptides with a first cleavage agent to generate polypeptide fragments;
 - (b) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented;
 - (c) identifying the isolated fragments by mass spectrometry;
 - (d) repeating steps (a)-(c) on the sample using a second cleavage agent that cleaves at a different site from the first cleavage agent; and
 - (e) characterising the polypeptides in the sample from the fragments identified in steps (c) and (d).
2. A method according to claim 1, wherein the step (d) comprises repeating steps (a)-(c) two or more times, each time using a further cleavage agent that cleaves at a different site from the previous cleavage agents.
3. A method according to claim 1 or claim 2, comprising a further capping step prior to step (a), which capping step comprises reacting the polypeptides in the sample with one or more capping agents to introduce capping groups on one or more reactive side chains of the polypeptides.
4. A method according to claim 3, wherein the capping step and steps (a)-(c) are repeated one, two, or more times, each time introducing capping groups at the same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

AMENDED SHEET

5. A method for characterising a polypeptide or a population of polypeptides, which method comprises:

- (f) contacting a sample comprising one or more polypeptides with a first capping agent in a first capping step to introduce capping groups on one or more reactive side chains of the polypeptides;
- (g) contacting the sample with a cleavage agent to generate polypeptide fragments;
- (h) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the polypeptide from which it was fragmented;
- (j) identifying the isolated fragments by mass spectrometry;
- (k) repeating steps (f)-(j) on the sample using a second capping agent that introduces capping groups at the same side chains as the first capping step, but uses capping groups having different mass than the capping groups used in the first capping step; and
- (l) characterising the one or more polypeptides in the sample from the fragments identified in steps (j) and (k).

6. A method according to claim 5, wherein the steps (f)-(j) are repeated two or more times, each time introducing capping groups at the same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

7. A method according to claim 5 or claim 6, wherein the step (k) comprises repeating steps (f)-(j) one, two, or more times, each time using a further cleavage agent that cleaves at a different site from the previous cleavage agents.

8. A method according to any of claims 3-7, wherein the side chains to be capped comprise one or more of the following:

- the NH₂ side chain in arginine;
- the NH₂ side chain in asparagine;
- the NH₂ side chain in glutamine;
- the NH₂ side chain in lysine;
- the COOH side chain in aspartic acid;
- the COOH side chain in glutamic acid;
- the OH side chain in serine;
- the OH side chain in threonine;
- the OH side chain in thyroxine;
- the OH side chain in tyrosine; and
- the SH side chain in cysteine.

9. A method according to any preceding claim, wherein the fragments are isolated by capture on a solid phase, such as DITC glass or polystyrene isothiocyanate.

10. A method according to claim 9, wherein the capture involves covalently bonding the fragments to the solid phase. —

11. A method according to claim 10, wherein the fragments are bound to the solid phase through their N-termini. —

12. A method according to any preceding claim, wherein each isolated fragment comprises the C-terminus of the polypeptide from which it was fragmented.

13. A method according to any preceding claim, wherein the cleavage agent employed comprises an endopeptidase or a chemical cleavage agent.

DRAFTS-4-021993

14. A method according to claim 13, wherein the cleavage agent employed comprises Lys-C endopeptidase, a thiocyanate compound, cyanogen bromide, BNPS-skatole, trypsin, chymotrypsin, and/or thrombin.

15. A method according to any of claims 3-14, wherein the capping agent comprises one or more of an iodoacetate compound, an isocyanate compound, a silyl compound, an anhydride, a vinylsulphone compound and a vinyl pyridine derivative.

16. A method for determining the expression of one or more proteins in a tissue, which method comprises characterising a population of polypeptides according to a method as defined in any preceding claim.

17. A method for assaying for one or more specific polypeptides in a sample, which method comprises characterising a population of polypeptides according to a method as defined in any of claims 1-15, and determining the presence or absence of the one or more specific polypeptides from the presence or absence of one or more specific fragments corresponding to the polypeptides.

add
at